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Reconstituted Histone-DNA Complexes

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Reconstituted histone–DNA complexes

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Complexes of λdv 21 plasmid DNA with calf thymus histones are reconstituted in the presence and absence of urea, and, in the absence of urea, at 4 and 37 °C. It has been concluded previously on the basis of restriction nuclease digestion of complexes reconstituted in the presence of urea that the preferential binding of individual histones to certain DNA sequences is abolished, by specific histone–histone interactions, when a mixture of the four small histones is used (Steinmetz, Streeck & Zachau 1975 *Nature, Lond.* **258**, 447). This conclusion holds also for the other conditions tested here. An exception is the pair of arginine-rich histones (H3–H4) which appears to bind specifically when reconstituted in the absence of urea by a one step dialysis procedure and non-specifically when the salt is removed by a stepwise dialysis procedure. The subunits which are formed when the four small histones are present simultaneously are very similar to nucleosome core particles according to the fragment patterns obtained with micrococcal nuclease. Protection of 155, 285, 420, 560 etc. nucleotide pairs is observed. When histone H1 is present in addition to the four small histones a 175 nucleotide pair fragment is protected, but no 200 nucleotide pair fragment or multiples thereof were formed under our conditions.

Introduction

It is now well established that chromatin is composed of subunits which are called nucleosomes (for reviews see Elgin & Weintraub 1975; Allfrey *et al.* (eds) 1976). Nucleosome core particles contain two molecules each of the histones H2A, H2B, H3 and H4, and 140 nucleotide pairs of DNA. The nucleosomes themselves contain also histone H1 and, at least in chromatin from rat and mouse liver or calf thymus, approximately 200 nucleotide pairs of DNA.

In this paper our experiments on chromatin digestion with DNase II are briefly summarized and attempts at chromatin reconstitution are dealt with in some detail.

When nuclei or chromatin were incubated with DNase II, cleavage occurred not only between nucleosomes but also near a centre of symmetry of the nucleosomes; a cleavage pattern with 100, 200, 300 etc. nucleotide pairs of DNA was produced instead of the usual 200, 400, 600 etc. nucleotide pair pattern (Altenburger, Hörz & Zachau 1976). The accessibility of DNA in the centre of the nucleosomes depends on the presence of low concentrations of Ca^{2+} or Mg^{2+} or higher concentrations of Na^{+} (Altenburger *et al.* 1976; Steinmetz, Streeck & Zachau 1978). The temperature dependence of the degradation of chromatin by DNase II was also investigated (Greil, Igo-Kemenes & Zachau 1976). Removal of histone H1 from calf thymus chromatin with 0.6 M NaCl abolished the cleavage at the centre of the chromatin subunit even when DNase II digestion was carried out in the presence of Mg^{2+} (Steinmetz *et al.* 1978). When histone H1 was removed from mouse liver chromatin by the tRNA procedure (Varshavsky, Ilyin & Georgiev 1974) the same was observed. Moreover, on readdition of histone H1 the 100 nucleotide pair cleavage pattern could be restored (Altenburger 1977). Chromatin superstructures termed solenoids are observed in the presence of histone H1

and divalent cations (Finch & Klug 1976), that is under conditions where DNase II produces a 100 nucleotide pair cleavage pattern; in the absence of divalent cations or histone H1 neither the superstructure nor the 100 nucleotide pair pattern is seen. Although the experimental conditions of the two lines of research will have to be compared in detail it may be possible to consider DNase II a biochemical probe into certain aspects of the superstructure of chromatin.

Our first attempts at chromatin reconstitution were directed towards the question of the specificity of histone–DNA interactions. A model system was used which consisted of the DNA of the small plasmid λdv 21 and calf thymus histones. It was investigated to what extent the cleavage of λdv DNA at each of the five sites for the restriction nuclease from *Haemophilus influenzae* Rd (*Hin*d II) was influenced by individual histones or histone combinations. All histones when applied individually showed a preference for protecting two of the five sites against *Hin*d II cleavage while the mixture of the four small histones which constitute the nucleosome core bound to DNA strongly but without preference for any particular part of the λdv DNA. Apparently the histone–histone interactions within the nucleosome core abolish the specificity of the histone–DNA interactions (Steinmetz *et al.* 1975).

Work on the reconstitution of chromatin and chromatin subunits has found widespread interest and is being carried out in several laboratories (e.g. Oudet, Gross-Bellard & Chambon 1975; Boseley *et al.* 1976; Sollner-Webb, Camerini-Otero & Felsenfeld 1976; Yaneva, Tasheva & Dessev 1976). In recent work in our laboratory it was found that reconstitution of DNA with the four small histones yielded nucleosome cores which according to micrococcal nuclease digestion were spaced in a register of about 140 nucleotide pairs. A proper 200 nucleotide pair spacing could not be obtained even in the presence of histone H1 (Steinmetz *et al.* 1978). In the course of this work a number of reconstitution conditions were investigated, in order to reach more general conclusions with respect to the specificity of histone–DNA interactions. In the following the results of such experiments are described which are not reported elsewhere.

Results and discussion

Histone binding to λdv DNA

The outline of the reconstitution experiments is described in figure 1. λdv 21 DNA is linearized with *Hin*d III and mixed, under high salt conditions, with increasing amounts of histone H2A. Salt and, in some experiments, urea are removed by a stepwise dialysis procedure. The resulting histone–DNA complexes are cleaved with *Hin*d II under conditions which are just sufficient to completely cleave histone-free DNA. After phenol extraction the DNA samples are electrophoresed (figure 1*a*). It can be seen that with increasing histone:DNA ratios fragments C, E, and F′ are missing; the DNA appears in larger fragments instead. The gels are scanned (figure 1*b*) and quantitatively evaluated (figure 1*c*). With increasing amounts of H2A a highly preferential protection of the two left-hand cleavage sites of λdv 21 DNA is observed.

The influence of the presence of urea during the reconstitution process on the quality of histone–DNA complexes has been discussed by several authors (e.g. Paul & More 1973; Boseley *et al.* 1976; Sollner-Webb *et al.* 1976). In our model system the influence of urea is small when histone H2A is used (figure 2*c*, to be compared with figure 1*c*). It is not much bigger in the case of histone H1 (figure 2*a*, to be compared with figure 3 in Steinmetz *et al.* 1975).

When the reconstitution is carried out in the absence of urea but at 37 °C instead of 4 °C, preferential protection of the two left-hand cleavage sites is again found (figure 2*b*).

The binding of the pair of arginine-rich histones (H3 and H4) to linear λdv 21 DNA is fairly sensitive to the method of reconstitution. A strong preference for the two left-hand sites is observed on reconstitution by a one step dialysis in the absence of urea (figure 3*a*). The

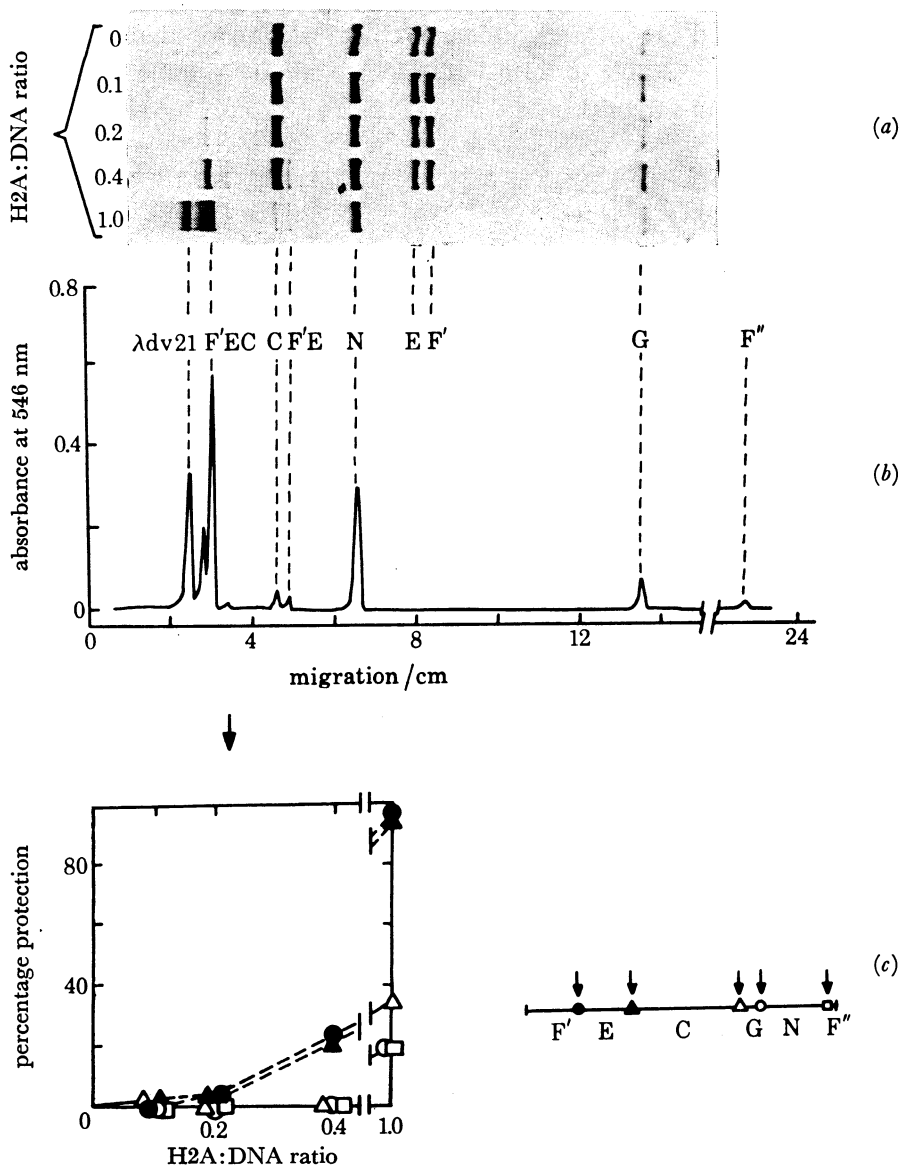

FIGURE 1. Protection of *Hin*d II cleavage sites in linear λdv 21 DNA by calf thymus histone H2A. (*a*) Complexes of linear λdv 21 DNA and histone H2A (0.1, 0.2, 0.4, and 1.0 mg H2A per milligram of DNA) were formed in salt-urea gradients, cleaved with *Hin*d II after addition of 0.1 volume of a tenfold concentrated standard buffer (Philippsen, Streeck & Zachau 1974), and the DNA fragments were analysed on a 4% polyacrylamide slab gel. For details of the reconstitution procedure, the restriction nuclease digestion and the gel electrophoretic separation see Steinmetz *et al.* (1975). (*b*) Densitometer tracing of the gel electrophoretic separation of DNA fragments obtained on digestion of the complex containing 1.0 mg H2A per milligram of DNA. (*c*) From the molar amounts of the DNA fragments the extent was calculated to which each of the five *Hin*d II cleavage sites in λdv 21 DNA was protected by histone H2A and plotted against histone:DNA ratio. The cleavage map of the linear λdv 21 DNA for *Hin*d II is also shown. The symbols in panels (*a*)–(*c*) are those used in the cleavage map.

preferential protection of these sites, however, is nearly abolished when complexes are formed using the salt–urea reconstitution procedure (figure 3*b*) and is no longer found in reconstitutes formed at 37 °C in the absence of urea by stepwise removal of the salt (figure 3*c*). In contrast to the individual histones the histone pair (H3–H4) therefore loses its ability to interact specifically with certain DNA sequences under some conditions and behaves similarly to the mixture of the four small histones. This may be relevant to the observation of Camerini-Otero, Sollner-Webb & Felsenfeld (1976) and Sollner-Webb *et al.* (1976) that the mixture of the arginine-rich histones alone is able to form with DNA a subnucleosome structure with some characteristics of chromatin subunits.

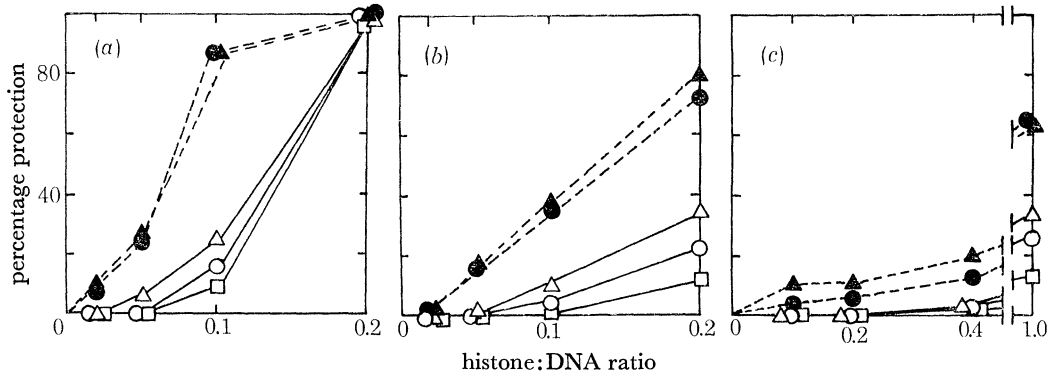

FIGURE 2. Protection of *Hin*d II cleavage sites in complexes reconstituted from linear λdv 21 DNA and calf thymus histone H1 or H2A using salt gradients without urea. For symbols and cleavage map see figure 1. (*a*) 50 μg of DNA were mixed with increasing amounts of histone H1 in 1 ml of buffer containing 2 M NaCl (buffer: 15 mM NaCl, 0.1 mM $CaCl_2$, 1 mM β-mercaptoethanol, 1 mM phenylmethylsulphonyl fluoride (PMSF), 1% isopropanol, 15 mM potassium phosphate, pH 7.3). After dialysis for 8 h at 4 °C against buffer containing 2 M NaCl, complexes were formed through stepwise removal of the salt by dialysis against solutions of the same buffer containing successively decreasing concentrations of NaCl (the time for each dialysis step is given in parentheses): 1.5 M (16 h), 1.0 M (8 h), 0.75 M (16 h), 0.5 M (8 h), 0.4 M (16 h), 0.1 M (8 h), and 15 mM (16 h). (*b*) Complexes of linear λdv 21 DNA and histone H1 were formed as described under (*a*) but the dialysis steps were carried out for 1 h each at 37 °C. (*c*) λdv 21 DNA was complexed with histone H2A using the reconstitution procedure outlined in (*a*).

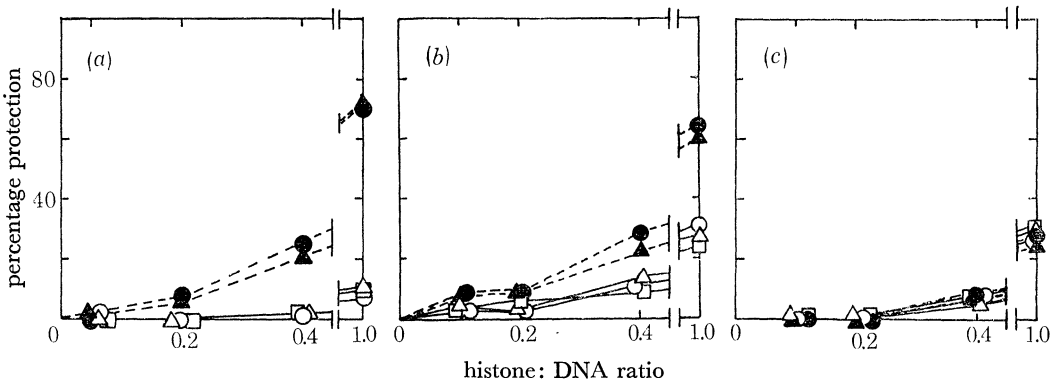

FIGURE 3. Protection of *Hin*d II cleavage sites in linear λdv 21 DNA by a mixture of calf thymus histones H3 and H4. For symbols and cleavage map, see figure 1. (*a*) 50 μg of linear λdv 21 DNA each were mixed with 2, 5, 10, 20, and 50 μg of an equimolar mixture of histones H3 and H4 in 650 μl of 2 M NaCl, 10 mM Tris–HCl (pH 8.0) and dialysed at 4 °C against 5 l of 0.5 mM EDTA, 10 mM Tris–HCl (pH 8.0). (*b*) (H3–H4)–DNA complexes were reconstituted in the presence of salt and urea as described in the legend to figure 1*a*. (*c*) Complexes were formed with (H3–H4) in the absence of urea as described in the legend to figure 2*b*.

Our earlier observation, that complexes which have been reconstructed from λdv 21 DNA and an equimolar mixture of the four small calf thymus histones no longer show the preferential protection exhibited by individual histones, holds for the various reconstitution procedures. All five *Hin*d II cleavage sites are protected nearly to the same extent in complexes formed either in the presence or absence of urea and, under the latter conditions, at 4 or 37 °C (figure 4). The slightly greater protection of the two left-hand sites may be due to incomplete formation of nucleosome core particles which will be discussed below.

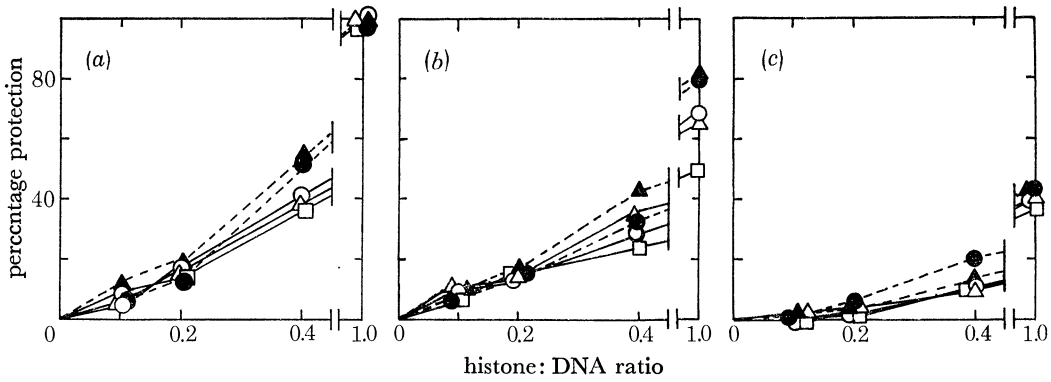

FIGURE 4. Protection of *Hin*d II cleavage sites in linear λdv 21 DNA by a mixture of the calf thymus histones H2A, H2B, H3, and H4. For symbols and cleavage map, see figure 1. Histone–DNA complexes were formed (*a*) by using the salt–urea procedure described in the legend to figure 1*a* (experiment also reported in Steinmetz *et al.* (1975), (*b*) in the absence of urea at 4 °C as described in the legend to figure 2*a*, and (*c*) in the absence of urea at 37 °C as outlined in the legend to figure 2*b*.

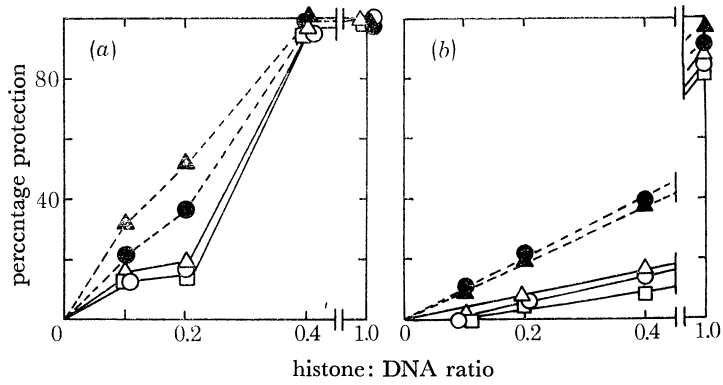

FIGURE 5. Protection of *Hin*d II cleavage sites in linear λdv 21 DNA by a mixture of the five calf thymus histones. For symbols and cleavage map, see figure 1. Histone–DNA complexes were reconstituted (*a*) in the presence of salt and urea as described in the legend to figure 1*a* (experiment also reported in Steinmetz *et al.* (1975)), and (*b*) in the absence of urea at 37 °C as described in the legend to figure 2*b*.

When histone H1, in addition to the four small histones, is included in the reconstitution mixture a more pronounced protection of all five cleavage sites is observed, regardless of the presence or absence of urea during the reconstitution procedure (figure 5). More important, however, is the finding that the preferential protection of the two left-hand cleavage sites reappears. This may be taken as an indication that a specific binding of histone H1, found also with histone-free DNA, is not abolished by histone–histone interactions.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

All three reconstitution procedures thus lead, except with (H3–H4), to the same result with respect to the specificity of histone–DNA interaction. Only small differences in the overall extent of protection of *Hin*d II cleavage sites were found in the three procedures. A direct comparison within one experimental series showed that a complex between DNA and the four small histones, reconstituted in the absence of urea, exhibited a somewhat less pronounced protection of the restriction sites than the one formed in the presence of urea (not shown).

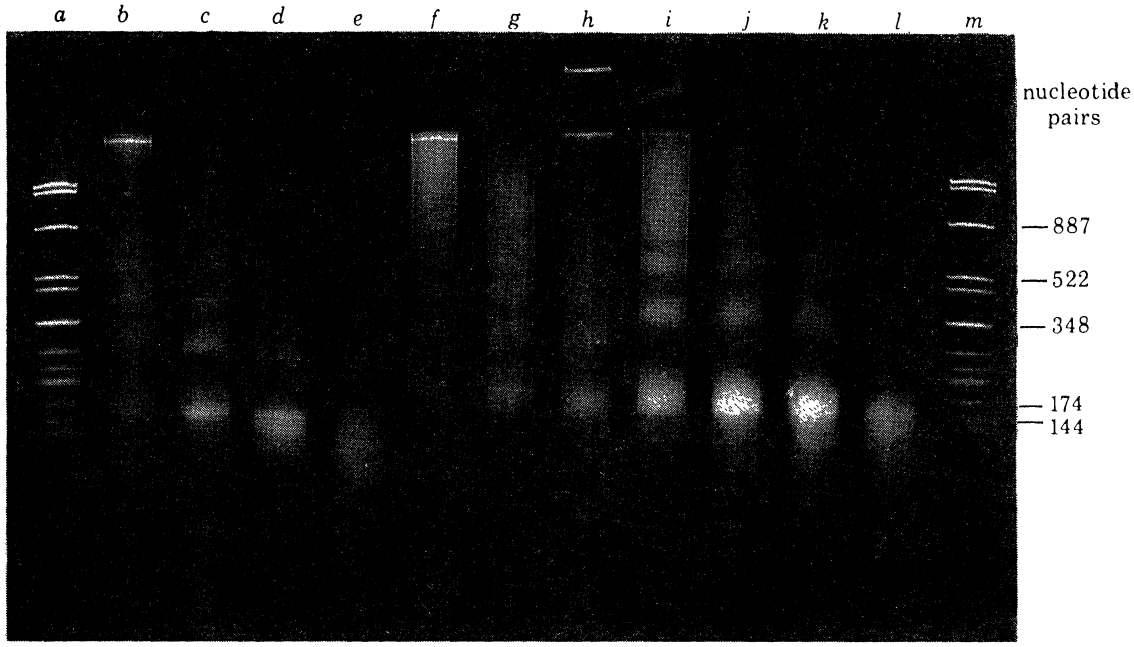

FIGURE 6. DNA fragments from micrococcal nuclease digests of rat liver nuclei and a complex reconstituted from λdv 21 DNA and a mixture of the four small calf thymus histones. The reconstituted complex which was prepared in the absence of urea at 37 °C (see legend to figure 2*b*) from supercoiled λdv 21 DNA (form I) and an equimolar mixture of the four small histones (histone: DNA ratio 1.25:1 by mass) was digested in the reconstitution buffer, with 2 units/ml of micrococcal nuclease for 15 s, 1, 5 and 30 min at 37 °C (*b–e*) and with 80 units/ml of micrococcal nuclease for 30 s, 5 and 30 min at 0 °C (*f–h*). Rat liver nuclei which were prepared by W. Pfeiffer according to Hewish & Burgoyne (1973) were incubated as described by Noll (1974) with 20 units/ml of micrococcal nuclease and 3 mM $CaCl_2$ for 2, 5, and 60 min at 37 °C (*i–l*). After the incubation DNA fragments were isolated and analysed on a 6% polyacrylamide slab gel (Weintraub, Palter & van Lente 1975) with a 4% stacking gel. For chain length determination of DNA fragments a Bsu digest of λdv 1 DNA (Steinmetz *et al.* 1978) was run in parallel (slots *a* and *m*). The gel was stained with ethidium bromide.

In general a comparison between different series of experiments is difficult due to possible differences in the extent of digestion. It should be noted that the degree of protection of a certain cleavage site in a particular complex should not be regarded as absolute but is relative to the degree of protection of the other sites in the same complex. The absolute values of the degree of protection at the various sites, and thus the differences between these values, may vary with the ionic conditions of the incubation mixture and the extent of the digestion.

In a separate series of experiments it was found that the supercoiled form of the λdv 21 DNA (form I) leads to the same results as the linear form (form III) of the DNA. When the supercoiled form was taken for reconstitution of histone–DNA complexes, a preferential protection of the two left hand cleavage sites by individual histones (H1 and H2A were tested) and an unspecific protection of all five cleavage sites by the mixture of the four small histones was

again found (not shown). (Only the salt–urea reconstitution procedure was employed in these experiments.)

The equal protection of all five cleavage sites by the mixture of the four small histones indicates that the subunits which have been formed under these conditions (see below) are randomly distributed along the DNA helix with respect to the five sites. This is in agreement with the results of Polisky & McCarthy (1975) and of Crémisi, Pignatti & Yaniv (1976) who found that nucleosome core particles are located randomly along SV40 DNA with respect to the EcoRI and Bam restriction nuclease cleavage sites.

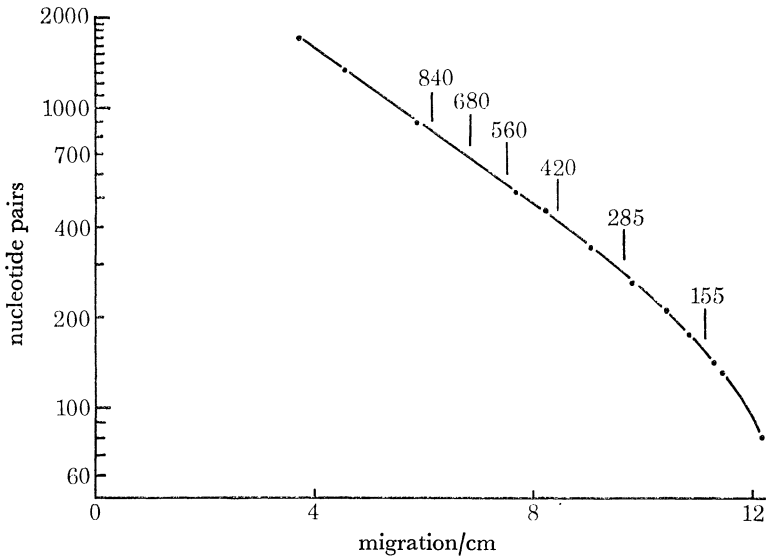

FIGURE 7. Calibration of DNA fragments from micrococcal nuclease digestion of the reconstituted complex. DNA fragments from a micrococcal nuclease digest (shown in Steinmetz *et al.* 1978) of the reconstituted complex were mixed with Bsu fragments of λdv 1 and subjected to electrophoresis in an agarose gel. Distances of migration were measured from the origin of the gel to the maxima of peaks in a densitometer tracing of the photograph of the gel. Sizes of Bsu restriction fragments of λdv 1 (Steinmetz *et al.* 1978) were plotted against migration distance and the points connected by a smooth curve. Vertical lines indicate the positions of the micrococcal nuclease fragments of the reconstituted complex. The calculation of the unit size from the length of the fragments is given in Steinmetz *et al.* (1978).

Formation of nucleosome core particles

In order to verify that chromatin subunits were formed in our reconstitution experiments with λdv 21 DNA and the mixture of the four small calf thymus histones, we digested the complex with micrococcal nuclease. This enzyme is known to cut preferentially between chromatin subunits, thereby giving rise to a series of DNA fragments the sizes of which are multiples of a unit length (Noll 1974; van Holde *et al.* 1974). Figure 6*b–h* shows the gel electrophoretic separation of DNA fragments obtained from a complex of λdv 21 DNA (form I) and a mixture of the four small histones by digestion with micrococcal nuclease at 0 and 37 °C for increasing lengths of time. For comparison fragments from micrococcal nuclease digests of rat liver nuclei were run in parallel on the same gel (figure 6*i–l*). It is obvious that the DNA fragments obtained from the reconstituted complex correspond to a repeat size considerably shorter than the one found in rat liver nuclei. Calibration of the DNA fragments from the reconstituted complex with admixed λdv 1–Bsu marker fragments (figure 7) yielded a unit

length of 137 ± 7 nucleotide pairs. The size of the repeat unit of the reconstituted complex is in good agreement with the length of the DNA in nucleosome core particles which have been obtained from chromatin by extensive digestion with micrococcal nuclease and which contain 140 nucleotide pairs of DNA (e.g. Shaw *et al.* 1976; Whitlock & Simpson 1976; Noll & Kornberg 1977). This suggests that an array of nucleosome core particles has been formed in our reconstitution experiment. Further evidence is gained from the patterns of 'subnucleosomal' DNA fragments which are similar in micrococcal nuclease digests of nuclei and the reconstituted complex (figure 6) as well as by DNase I digestion experiments (Steinmetz *et al.* 1978).

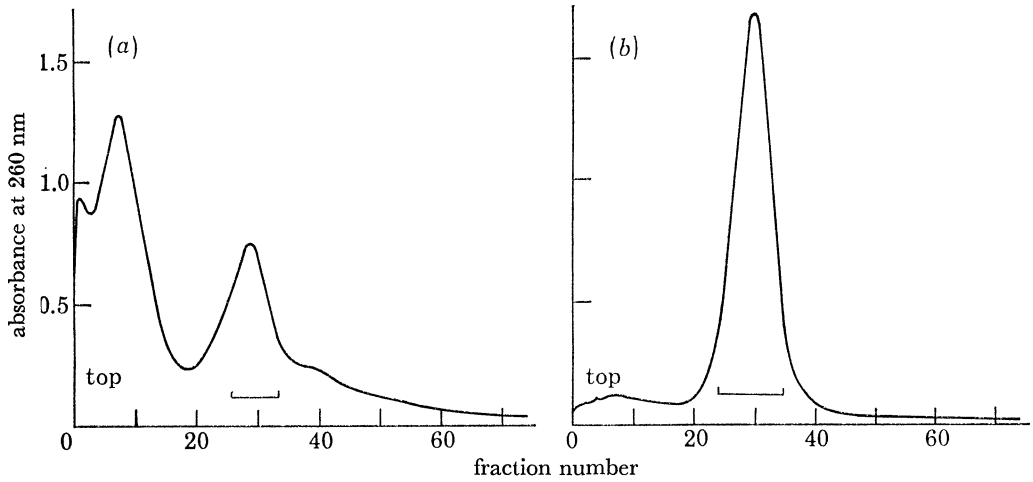

FIGURE 8. Isolation of subunits of a reconstituted complex by sucrose gradient fractionation of a micrococcal nuclease digest. Complexes were reconstructed at 4 °C from 10 mg of calf thymus DNA (Boehringer Mannheim) and 9 mg of an equimolar mixture of the four small histones using the reconstitution procedure outlined in the legend to figure 2*a*. Digestion was carried out at a concentration of 1 A_{260} unit/ml with 1 unit per ml of micrococcal nuclease for 17 min at 30 °C (1 A_{260} unit is the amount of material in 1 ml which has an absorbance of 1.0 at 260 nm in a 1 cm light path). The reaction was stopped with EDTA (1 mM final concentration). The digested complexes were dialysed against 1 mM Tris–HCl, 1 mM EDTA, 1 mM PMSF, 1% isopropanol (pH 7.8) and concentrated to about 7 A_{260} units/ml using dry Sephadex G-100. (*a*) Aliquots (2 ml) of the concentrated solution were layered on a 34 ml isokinetic sucrose gradient (McCarty, Vollmer & McCarty 1974) with $c_{mix} = 5\%$, $c_r = 28.8\%$ and $v_m = 201$ ml in 1 mM Tris–HCl, 1 mM EDTA, 1 mM PMSF, 1% isopropanol (pH 7.8), and centrifuged at 24 h at 27000 rev/min and 3 °C in a Beckman SW 27 rotor. The gradient was monitored for absorbance by pumping it through a turbulence-free flow cell. Fractions of 0.5 ml were collected; fractions containing the monomer were pooled as indicated, dialysed, and further purified. (*b*) 8 A_{260} units of the monomer fraction from the first sucrose gradient were centrifuged through a second sucrose gradient identical to the first.

Additional evidence that the reconstitution leads to core particles comes from the isolation of the subunit of the reconstituted complex after partial micrococcal nuclease digestion (figure 8). The monomer particle contains about equal amounts of the four histones, a DNA fragment of about 140 nucleotide pairs, and has a sedimentation coefficient of 11.6*S* (Steinmetz *et al.* 1978). When the monomer from the reconstituted complex was analysed by low-angle X-ray scattering by A. Tardieu (Centre de Génétique Moléculaire, C.N.R.S., Gif-sur-Yvette) the spectrum obtained was slightly different from the one of nucleosome core particles from chromatin at high angles but quite similar at low angles. We conclude that reconstitution of DNA with the four small calf thymus histones led to formation of particles which in most respects are identical to nucleosome cores.

Reconstitution experiments with a mixture of all five calf thymus histones did not give rise to the 200 nucleotide pair periodicity found in calf thymus chromatin. Again, core subunits were obtained as indicated by the appearance of a series of DNA fragments with a unit length of about 140 nucleotide pairs upon micrococcal nuclease digestion (figure 9). The background of DNA fragments throughout the gel was much higher than in the corresponding experiments without histone H1 (figure 9). In addition, a stable prominent DNA fragment of about 175

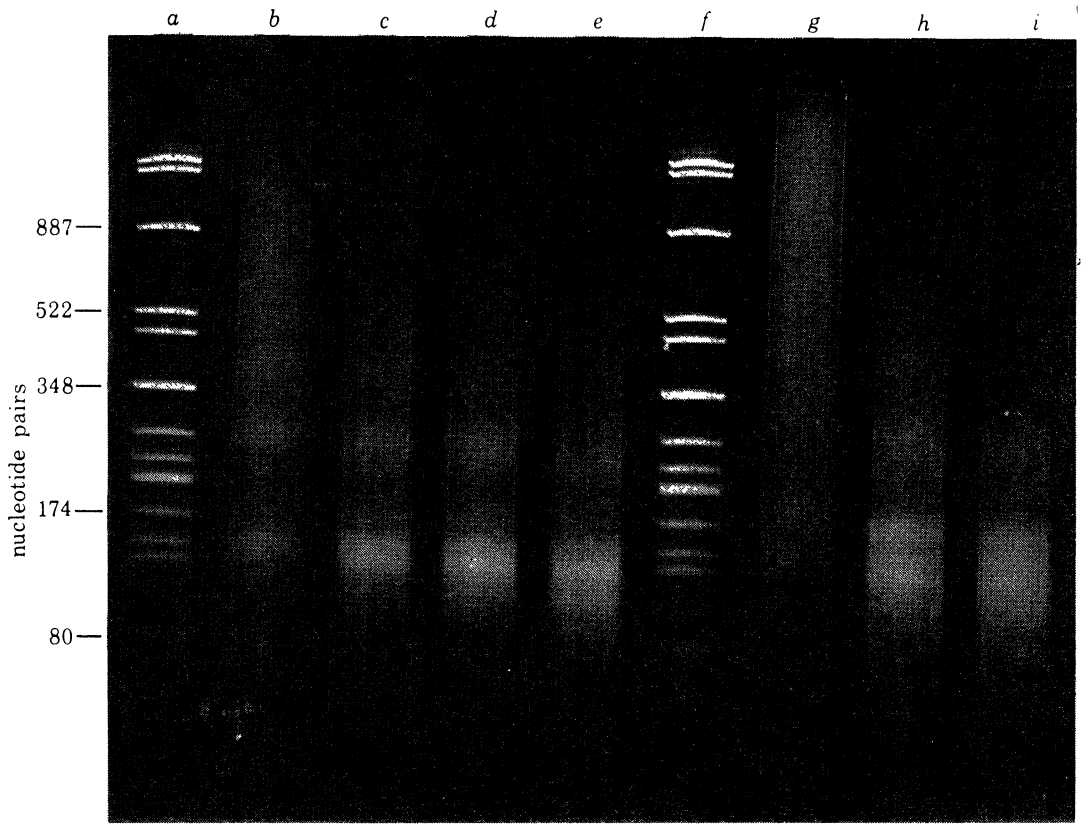

FIGURE 9. Protection of a 175 nucleotide pair fragment in complexes containing H1 in addition to the four small histones. Complexes were reconstructed in the absence of urea at 4 °C (see legend to figure 2*a*) from λdv 21 DNA (form I) and an equimolar mixture of the four small calf thymus histones (histone:DNA ratio 1.5:1 by mass) as well as a mixture of all five calf thymus histones (H1:(H2A–H2B):(H3–H4):DNA = 0.1:0.5:0.5:1 by mass). Micrococcal nuclease digestion was carried out at 37 °C with 2 units/ml of enzyme for 1, 3, 5 and 10 min (complexes without H1, *b–e*), and for 1, 5 and 30 min (complexes containing H1, *g–i*). The resulting DNA fragments (aliquots of 0.1 A_{260} units each) were subjected to gel electrophoresis as described in the legend to figure 6. Slots *a* and *f* contained λdv 1 – Bsu fragments as size standards.

nucleotide pairs was produced in the course of the digestion which was not found in digests of complexes lacking histone H1 (figure 9). A fragment of similar size had also been found in digests of chromatin and reconstitutes by other workers to be dependent on the presence of histone H1 (Axel, Melchior, Sollner-Webb & Felsenfeld 1974; Camerini-Otero *et al.* 1976). This fragment may be due to the binding of histone H1 to separately located, i.e. not tandemly arranged, core subunits and protection of an extra segment of 20–35 nucleotide pairs.

Concluding remarks

Cleavage of histone–λdv DNA complexes with a restriction nuclease has proven to be a useful method for investigating the specificity of histone–DNA interactions. The structural reasons for the preferential binding of histones to certain DNA sequences are not yet clear, but the difference between the binding behaviour of individual histones and all four of the histones present in nucleosome cores is a general observation which holds for a number of different reconstitution conditions. Clearly, complexes consisting of individual histones and DNA do not reflect the binding behaviour of histones in chromatin.

The method of restriction nuclease cleavage of histone–DNA complexes monitors the protection of the specific nuclease cleavage sites and suggests that these sites are covered randomly by nucleosome cores. To decide whether the nucleosome cores are distributed over the whole DNA molecule in a completely random or in a partially non-random fashion would require the analysis of the protection at several sets of closely spaced cleavage sites.

The method also gives some clues as to the binding of histone H1 when present in addition to the four small histones forming the nucleosome core, although here digestion with non-specific nucleases such as micrococcal nuclease may be more valuable. It shows that the main limitation of our reconstitution procedure at present is the inability to reconstruct tandemly arranged nucleosomes containing all five histones and about 200 nucleotide pairs of DNA as found in calf thymus and other chromatins. It is possible that histone modifications are important for the correct assembly of nucleosomes *in vivo* and *in vitro*.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

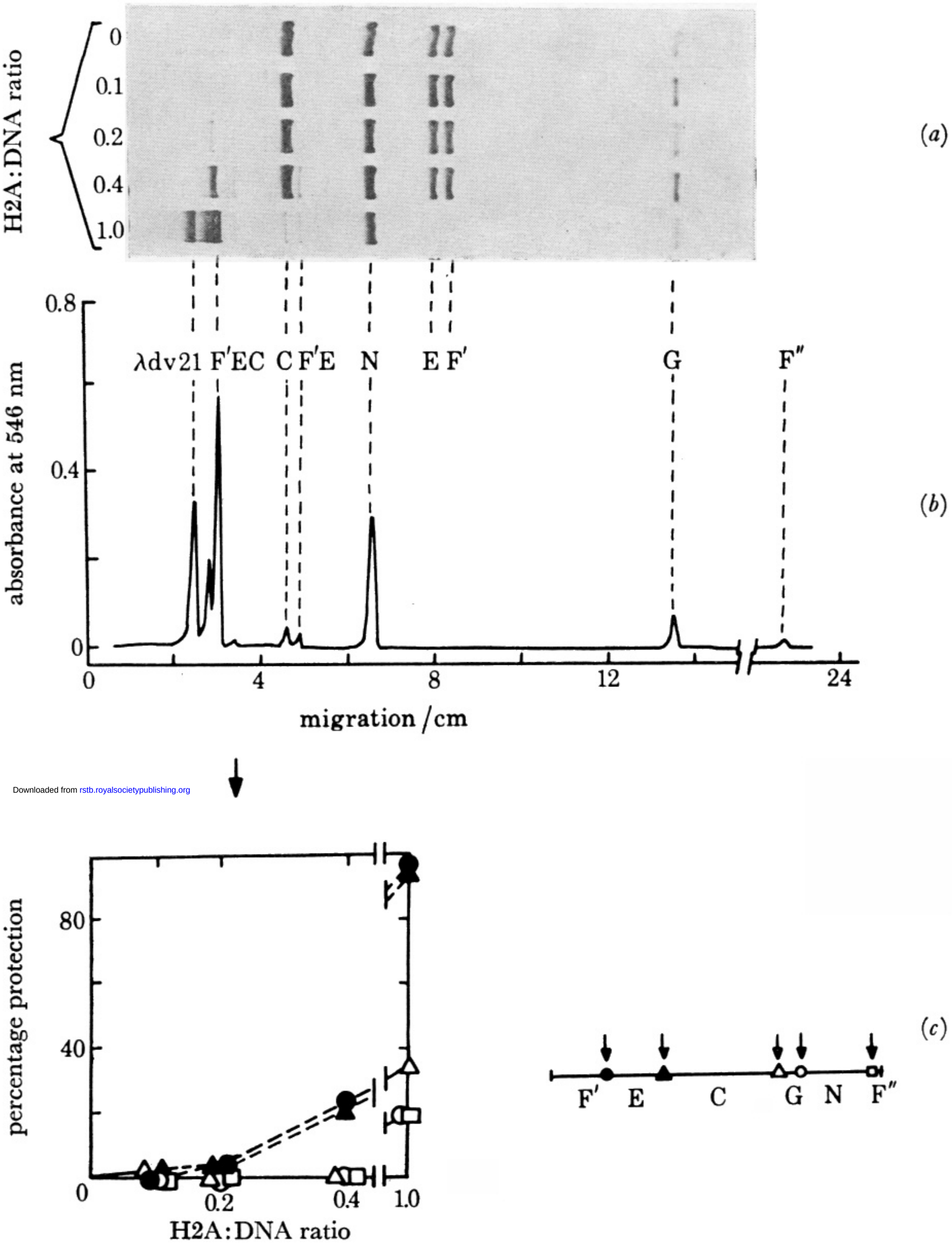

FIGURE 1. Protection of *Hin*d II cleavage sites in linear λdv 21 DNA by calf thymus histone H2A. (*a*) Complexes of linear λdv 21 DNA and histone H2A (0.1, 0.2, 0.4, and 1.0 mg H2A per milligram of DNA) were formed in salt-urea gradients, cleaved with *Hin*d II after addition of 0.1 volume of a tenfold concentrated standard buffer (Philippsen, Streeck & Zachau 1974), and the DNA fragments were analysed on a 4% polyacrylamide slab gel. For details of the reconstitution procedure, the restriction nuclease digestion and the gel electrophoretic separation see Steinmetz *et al.* (1975). (*b*) Densitometer tracing of the gel electrophoretic separation of DNA fragments obtained on digestion of the complex containing 1.0 mg H2A per milligram of DNA. (*c*) From the molar amounts of the DNA fragments the extent was calculated to which each of the five *Hin*d II cleavage sites in λdv 21 DNA was protected by histone H2A and plotted against histone:DNA ratio. The cleavage map of the linear λdv 21 DNA for *Hin*d II is also shown. The symbols in panels (*a*)–(*c*) are those used in the cleavage map.

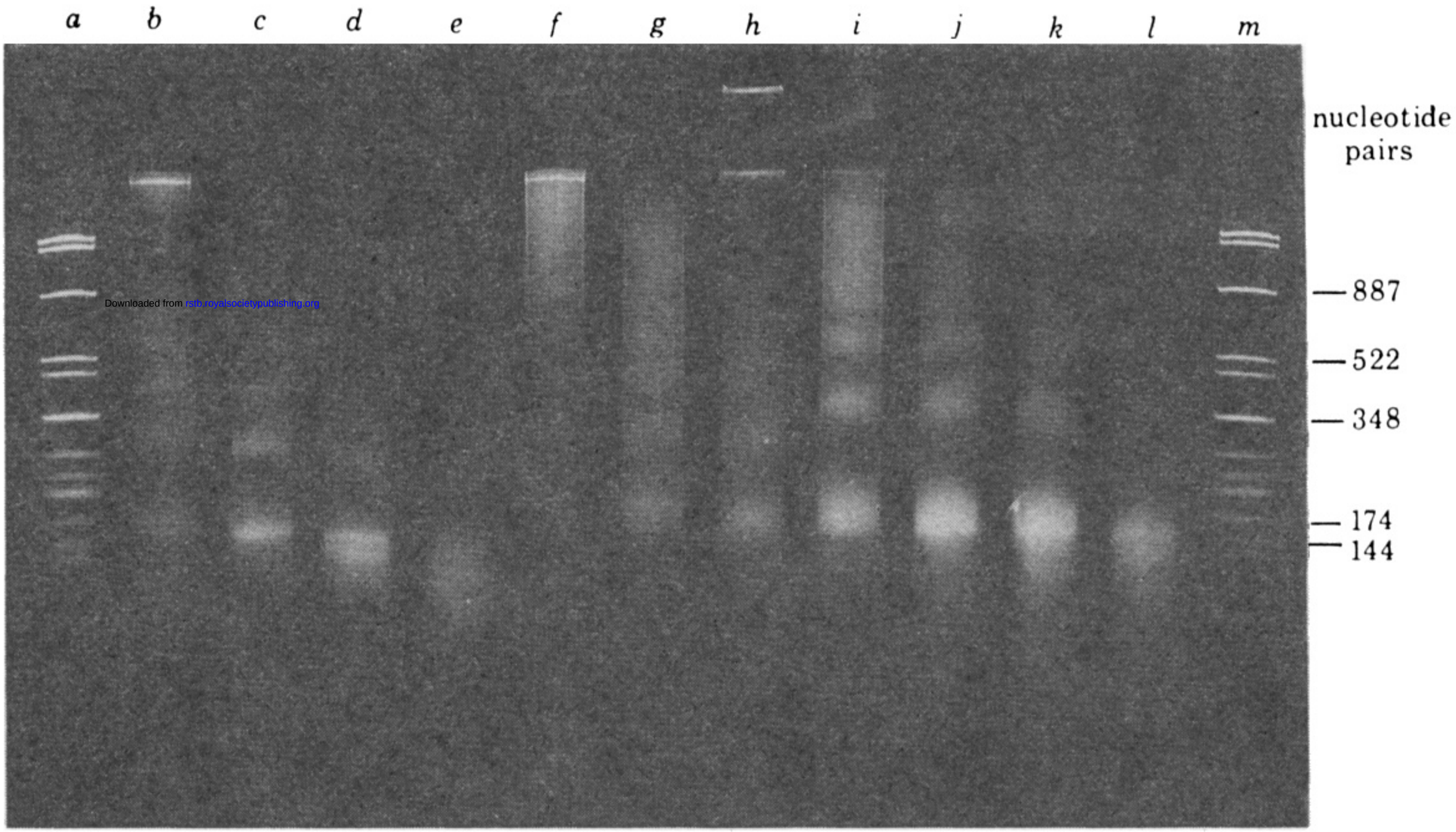

FIGURE 6. DNA fragments from micrococcal nuclease digests of rat liver nuclei and a complex reconstituted from λdv 21 DNA and a mixture of the four small calf thymus histones. The reconstituted complex which was prepared in the absence of urea at 37 °C (see legend to figure 2*b*) from supercoiled λdv 21 DNA (form I) and an equimolar mixture of the four small histones (histone: DNA ratio 1.25:1 by mass) was digested in the reconstitution buffer, with 2 units/ml of micrococcal nuclease for 15 s, 1, 5 and 30 min at 37 °C (*b–e*) and with 80 units/ml of micrococcal nuclease for 30 s, 5 and 30 min at 0 °C (*f–h*). Rat liver nuclei which were prepared by W. Pfeiffer according to Hewish & Burgoyne (1973) were incubated as described by Noll (1974) with 20 units/ml of micrococcal nuclease and 3 mM $CaCl_2$ for 2, 5, and 60 min at 37 °C (*i–l*). After the incubation DNA fragments were isolated and analysed on a 6% polyacrylamide slab gel (Weintraub, Palter & van Lente 1975) with a 4% stacking gel. For chain length determination of DNA fragments a Bsu digest of λdv 1 DNA (Steinmetz *et al.* 1978) was run in parallel (slots *a* and *m*). The gel was stained with ethidium bromide.

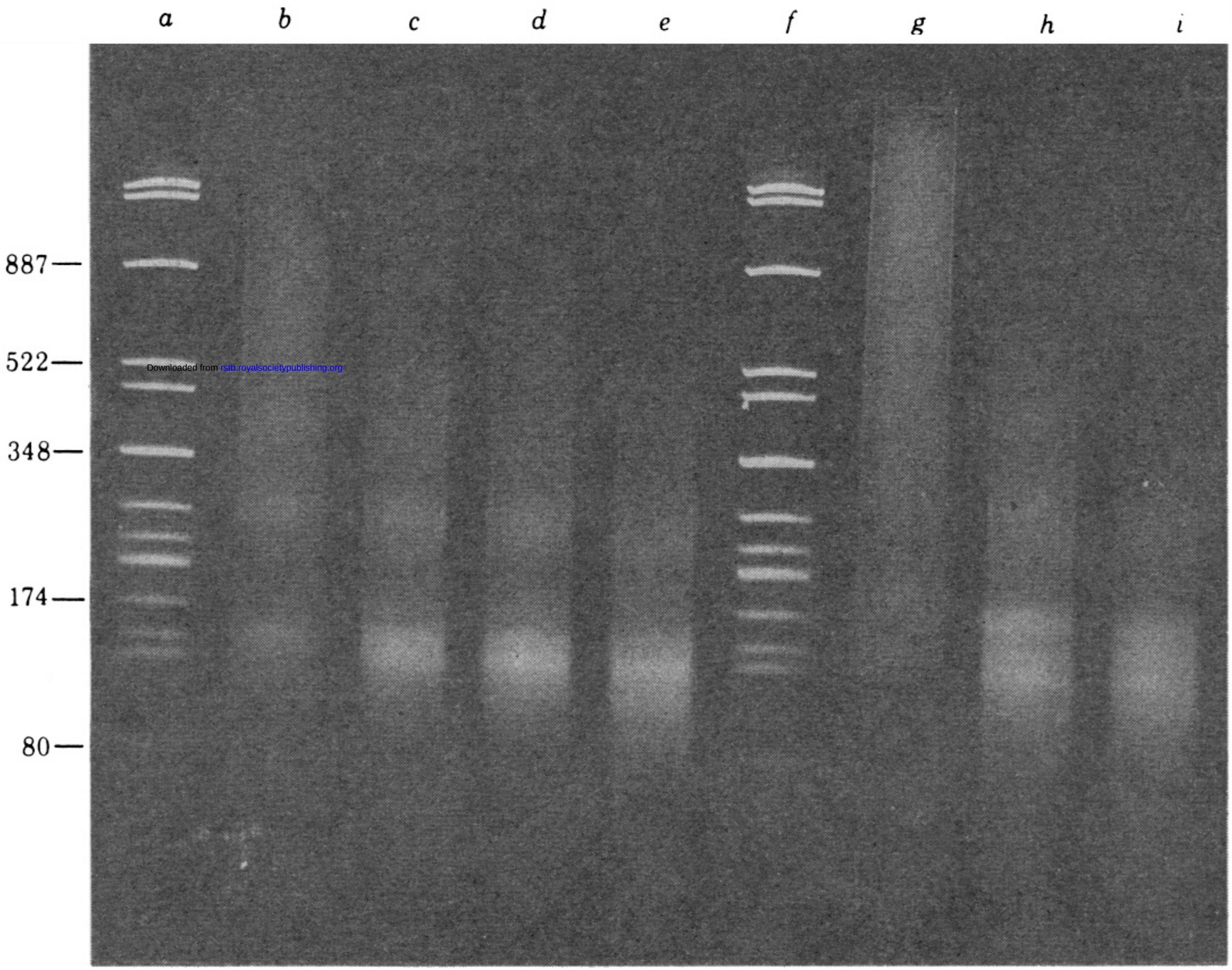

FIGURE 9. Protection of a 175 nucleotide pair fragment in complexes containing H1 in addition to the four small histones. Complexes were reconstructed in the absence of urea at 4 °C (see legend to figure 2*a*) from λdv 21 DNA (form I) and an equimolar mixture of the four small calf thymus histones (histone:DNA ratio 1.5:1 by mass) as well as a mixture of all five calf thymus histones (H1:(H2A–H2B):(H3–H4):DNA = 0.1:0.5:0.5:1 by mass). Micrococcal nuclease digestion was carried out at 37 °C with 2 units/ml of enzyme for 1, 3, 5 and 10 min (complexes without H1, *b*–*e*), and for 1, 5 and 30 min (complexes containing H1, *g*–*i*). The resulting DNA fragments (aliquots of 0.1 A_{260} units each) were subjected to gel electrophoresis as described in the legend to figure 6. Slots *a* and *f* contained λdv 1 – Bsu fragments as size standards.